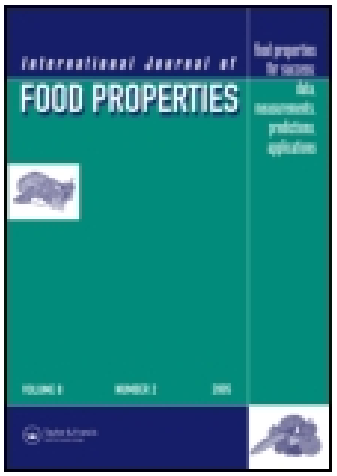

International Journal of Food Properties
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ljfp20

Prediction of Phenolic Composition of Monastrell and Tempranillo Wines: Correlation between Phenolic Content and Traditional Variables of Fruit Maturity

N. Martí[a], V. Lizama[b], J.A. Verdú[a], N. Muñoz[c], J.L. Aleixandre[b] & D. Saura[a]

[a] IBMC-JBT Corp. FoodTech R&D Alliance, Instituto de Biología Molecular y Celular (IBMC), Universidad Miguel Hernández, Elche, Alicante, Spain

[b] Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Universidad Politécnica de Valencia, Valencia, Spain

[c] Mitra Sol., S.L. CEEIM Building, Universidad de Murcia, Campus de Espinardo, Murcia, Spain

Accepted author version posted online: 06 Aug 2012.Published online: 17 Dec 2014.

To cite this article: N. Martí, V. Lizama, J.A. Verdú, N. Muñoz, J.L. Aleixandre & D. Saura (2015) Prediction of Phenolic Composition of Monastrell and Tempranillo Wines: Correlation between Phenolic Content and Traditional Variables of Fruit Maturity, International Journal of Food Properties, 18:3, 465-479, DOI: 10.1080/10942912.2011.570467

To link to this article: http://dx.doi.org/10.1080/10942912.2011.570467

International Journal of Food Properties, 18:465–479, 2015

ISSN: 1094-2912 print/1532-2386 online
DOI: 10.1080/10942912.2011.570467

Prediction of Phenolic Composition of Monastrell and Tempranillo Wines: Correlation between Phenolic Content and Traditional Variables of Fruit Maturity

N. Martí[1], V. Lizama[2], J.A. Verdú[1], N. Muñoz[3], J.L. Aleixandre[2], and D. Saura[1]

[1]*IBMC-JBT Corp. FoodTech R&D Alliance, Instituto de Biología Molecular y Celular (IBMC), Universidad Miguel Hernández, Elche, Alicante, Spain*
[2]*Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Universidad Politécnica de Valencia, Valencia, Spain*
[3]*Mitra Sol., S.L. CEEIM Building, Universidad de Murcia, Campus de Espinardo, Murcia, Spain*

Color is one of the most important factors in the quality assessment of red wine. Polyphenolic compounds, located in the skin and seeds of grapes, are responsible for the color of both the fruit and the wine produced from that fruit. In the present research, the level of phenolic maturity of Monastrell and Tempranillo grapes at their time of arrival to the winery was investigated. To this end, parameters defining color and polyphenol content in wines produced from these grapes were contrasted with parameters typically associated with general and phenolic grape maturity. The main aim of this research was to uncover the nature of the relationship between these parameters, such that, by knowing the characteristics of the grapes, predictions could be made regarding the characteristics of the resulting wine. The capacity to objectively assess and predict the potential quality and enological fitness of a given lot of Monastrell and Tempranillo grapes would greatly facilitate selection of the style of vinification best suited to this fruit.

Keywords: Polyphenols, Extractability, Monastrell, Tempranillo.

INTRODUCTION

During grape ripening, berries increase in volume and sugar content, while declining in acidity level due to reaction and dilution of malic and tartaric acids in the system, as well as to the accumulation of potassium. These parameters are measures of the degree of maturity of the pulp.[1,2] Pulp maturity can be characterized by the following traditional maturity factors: weight of 100 grape berries, °Brix, pH, and total acidity. However, such analyses of maturation only give us information about the maturity of the pulp, not about that of the skin or seeds. This information gap can be filled by

Received 16 September 2010; accepted 3 March 2011.
Address correspondence to Nuria Martí, IBMC-JBT Corp. FoodTech R&D Alliance, Instituto de Biología Molecular y Celular (IBMC), Universidad Miguel Hernández, Avenida de la Universidad s/n, E-03202 Elche, Alicante, Spain. E-mail: nmarti@umh.es

the introduction of the concept of *phenolic maturity*, which not only considers the total content of polyphenolic compounds, but also takes into account as a component of this maturity how readily extractable these compounds are from grape skin and seeds.[3,4]

As the maturation process advances, the concentration of anthocyanins and tannins in the grape skin increases, while simultaneously, skin cell membranes begin to undergo degradation, with the result that the latter process facilitates extraction of the former compounds during vinification. The seeds, at this stage, will have reached a relatively low concentration of astringent tannins.[5–7] Rio Segade et al.[8] discuss research on grape texture properties and their relationship to phenolic ripeness and extractability.

The research presented in this article is divided into two parts. The first part explored correlations between classical variables of maturity and those which define polyphenolic maturity in order to: (1) evaluate the nature of their relationship; and (2) consider whether or not traditional parameters provide a sufficient basis for predicting the phenolic content and degree of phenolic maturity of grapes. In the second part of the research, analyses of the information obtained from the study of the correlation between the variables corresponding to phenolic compounds in grapes, as proposed by the Lamadon[9] method, and variables related to the evaluation of polyphenolic maturity through the Saint Cricq de Gauleac[4] method, are presented. The aim of this second part was to investigate the relationship between variables that measure similar parameters in order to determine the equivalence between prediction methods for the chromatic features of wine.

The values of the analytical parameters in grapes depend on a large number of factors (variety, genotype, climate, soil, cultural practices, etc.),[10] some of which are essentially random, and as such, cannot be determined or predicted accurately. Statistical regression techniques are essential when it is not possible to previously determine the values of the independent variables, as it is otherwise impossible to design an experiment that guarantees the orthogonality of the points to be investigated.[11]

MATERIALS AND METHODS

Seven manually harvested batches of grapes from the Bodega Cooperativa del Campo San Blas de Sax (Alicante)—three of Monastrell and four of Tempranillo—were used for this study. The batches were made up of different samples collected from the trucks as they arrived at the winery. The grape samples that composed each batch were generally harvested from distinct lots maintained by distinct growers, but did have a common harvest date. Across the batches of Monastrell[4] and Tempranillo[7] grapes, the harvest dates were different, but all seven batches were derived from the same growing region and were, therefore, assumed to have developed and ripened under common climatic conditions.

The Monastrell and Tempranillo grapes were hand harvested into 15-kg baskets, which were subsequently emptied into 1000- to 3000-kg trailers for transport to the winery. Once at the winery, and after collecting routine data on probable degree of alcohol, acidity, and °Brix, samples were drawn to carry out the analyses requisite to this study. Grape samples were crushed and destemmed using a horizontal destemmer-crusher with steel blades (slow turn speed) and a press with rubber rollers. The destemmed, crushed grapes were passed through a piston pump into 400hL tanks adjusted to 28°C by a temperature control system. An addition of 50 mg/L SO_2 was made to all tanks. Acidity was adjusted according to variety, with an addition of 0.5 g/L tartaric acid to each Monastrell tank (M1, M2, and M3) and 1 g/L to each Tempranillo tank (T1, T2, T3, and T4). Yeast and ammonium phosphate (30 g/hL), as a nitrogen source, were also added. Pectolytic enzymes (2.5 g/hL, *Rapidase Ex-Color*) were added initially, once the must was added to the tank, where it received pump-overs daily for 8 days for color and aroma extraction. Afterwards, the skins were pressed off and the free run and press wine were decanted into a tank so that alcoholic and malolactic fermentations could

run to completion. All the wines were produced in the same way so as to not introduce additional sources of variability beyond those being considered in this study.

Analysis of Grapes

The parameters of traditional maturity measured here were weight of 100 grape berries, total acidity, and °Brix (Table 1).[12] During fermentation monitoring, density, volatile acidity, total and free SO_2, and reducing sugars were measured and recorded (Table 2). The concentration of anthocyanins was determined through decolorization by SO_2,[13] and the concentration of potential of total extractable anthocyanins (IPT) by measuring absorbance at 280 nm of a sample of wine previously diluted 101 times in distilled water.

Phenolic compounds were analyzed from samples of 300 crushed grapes. The following were determined: total anthocyanins and phenolic compounds,[9] IPT, and the ratios for cellular extractability of anthocyanins and seed maturity.[4] Both methods used, Lamadon and Saint-Cricq, are based on differences in the extraction of polyphenolic compounds under varied conditions: with alcohol present, in an acid solution (pH 1.0), or in a pH 3.2 solution. All analyses were performed two times with three replicates each time, and the average of the results obtained were used for subsequent calculations.

Analysis of Wines

The gelatin index is a measure of reactions between wine tannins and gelatin proteins. Practically, this reactivity corresponds to the sensation of astringency of red wine in the mouth.[14] The method is based on the chemical properties of tannins that favor their reaction with proteins to form stable complexes. The condensed tannins present in wine precipitate homogeneously and reproducibly with the gelatin.[14] These tannins are basically made up of small polymers of procyanidins and other specific condensed molecules.[15,16] Experimentally, samples are prepared from two 50-mL volumes of wine. To one is added 5 mL of gelatin dissolved in water to a concentration of 70 g/L, and the same quantity of pure water is added to the other (control). The samples are left to precipitate for 3 days at 4°C of temperature, then centrifuged, and the tannins in both samples are calculated based on the total polyphenol index (TPI).[11]

The TPI of the wines was ascertained by measuring the optical density (DO280) of a sample of wine, diluted 101 times with distilled water, against pure distilled water, at 280 nm, with a 10-mm path length and quartz cuvette.[14] Measurement of color intensity was carried out by spectrophotometer, obtaining the optical density at longitudinal wavelengths of 420 and 520 nm,

TABLE 1
Results obtained from the analysis in grape

	Lamadon method		*Saint-Cricq de Gaulejac et al. method*					
Sample	*ANTLAM (g/Kg)*	*CFTLAM (g/Kg)*	*ANTpH1*	*ANTpH3*	*IPTpH1*	*IPTpH3*	*EA*	*Mp*
T1	934.5	4.008	780.5	418.3	43.2	29.0	46.4	42.3
T2	829.5	4.032	595.0	338.3	39.2	23.6	43.1	42.7
T3	1099.9	4.440	1090.3	500.5	47.2	31.0	54.1	35.4
T4	900.4	3.264	936.3	484.8	40.2	26.2	48.2	26.0
M5	601.1	2.256	735.0	297.5	36.4	24.0	59.5	50.4
M6	669.4	4.440	624.8	316.8	41.4	31.6	49.3	59.9
M7	1115.6	4.728	1165.5	390.3	53.8	27.4	66.5	43.0

TABLE 2
Results obtained from the analysis in wine

Sample	*Dens. (g/L)*	*Wine pH*	*Wine TA (g/L)*	*VA wine (g/L)*	*Free SO_2 (mg/L)*	*T SO_2 (mg/L)*	*Sugar (g/L)*	*Alc (%)*	*C.I. (*)*	*Tint (*)*	*Wine ANT (mg/L)*	*T.P.I. (*)*	*IND GEL (*)*
T1	0.9940	3.80	4.74	0.26	25.6	57.6	1.75	13.6	8.9	0.564	611.1	68.2	50.5
T2	0.9945	3.83	4.59	0.29	19.2	43.5	1.96	12.7	8.8	0.579	585.5	62.6	52.1
T3	0.9990	3.74	5.20	0.29	21.8	46.1	2.5	14.1	10.4	0.531	633.5	65.9	44.1
T4	0.9980	3.76	4.90	0.26	23.0	52.5	2.38	12.9	9.3	0.537	607.9	58.7	42.9
M5	0.9935	3.89	4.28	0.33	20.5	48.6	1.82	12.8	8.7	0.598	529.9	52.0	56.4
M6	0.9975	3.88	4.44	0.29	21.8	51.2	2.33	14.6	9.1	0.585	520.1	62.2	55.7
M7	0.9955	3.79	5.05	0.29	25.6	56.3	2.08	14.1	10.2	0.539	563.7	65.2	41.2

(*): Dimensionless magnitude.

with a 10-mm path length and glass cuvette. During wine aging, DO_{520} decreases, while DO_{420} is maintained; therefore, the tint value of wine continues to increase as wine ages.[12,13]

Statistical Analysis of Results

Statistical analysis was carried out using StatgraphicS Plus 5.0 for Windows (Statpoint Technologies, Inc. Warrenton, Virginia).[15] Linear correlations between the variables corresponding to traditional and phenolic maturity factors were studied to determine how the two sets of maturity parameters related to each other. These correlations, together with those derived by relating the traditional and phenolic variables to the corresponding phenolic and color parameters determined from the wines (dependent variables), proved very useful for obtaining early information about the variables that would form part of the multiple linear regression analysis. Because of the close relationship between organoleptic qualities and the commercial value of wine,[4,15] color parameters (tint and colorant intensity) and phenolic parameters (concentration of anthocyanins, polyphenol ratio, and gelatin ratio) were chosen as dependent variables. The variables corresponded to the analyses carried out on the grapes, which were classified into three groups, as seen in Table 3.

With respect to prediction of each of the wine parameters (concentration of anthocyanins, polyphenol ratio and gelatin ratio, tint, and color intensity), two distinct methods were used to generate two sets of linear regression equations. Variables in the first group of equations included the content of phenolic compounds in the grapes derived by using the Lamadon method.[9] In the second group, they included phenolic maturity values calculated using the Glories method,[8] and later developed by Saint-Cricq de Gaulejac et al.[4] For each group of equations the variables used, besides those belonging to each method, were those corresponding to the measures of traditional maturity.

When constructing multiple linear regression models, it is important to avoid introducing, conjointly, variables with a high level of inter-correlation. Models that do include highly inter-correlated variables, present two key problems. For one, the presence of high degrees of correlation between coefficients estimated by the equations makes it difficult, or even impossible, to calculate the real effect of each of the variables separately. In other words, the individual significance of each of the

TABLE 3
Explicative variables of the regression models and their abbreviations

Group	*Explicative variable*	*Key*
Traditional calculations of maturity	Weight of 100 grapes (g)	Weight/100
	Degrees Brix of must	BRIX
	pH of must	grape pH
	Total acidity of must (g/L)	grape TA
Calculations of phenolic compounds in grapes with the Lamadon method (1995)	Anthocyans in grapes (g/Kg)	ANTLAM
	Total phenolic compounds in grapes (g/Kg)	TPCLAM
Calculations of phenolic maturity according to the method by Saint-Cricq de Gaulejac et al.[4],	Total potential of anthocyans	ANTpH1
	Potential of extractable anthocyans	ANTpH3
	Total phenolic index	TPIpH1
	Total phenolic index (d_{280})	TPIpH3
	Extractability index of anthocyans	EA
	Phenolic maturity of seeds	Mp

variables remains undetermined. And secondly, due to the high degree of correlation between the variables, there is an increase in the error associated with coefficients of the model, with respect to the ideal model, in which variables do not correlate (orthogonal variables). To avoid this, variables that had a correlation higher than 0.6 were not included in the models; subsequently, resulting equations that presented coefficients with a correlation higher than 0.5 were also rejected (absolute values).

RESULTS AND DISCUSSION

Linear Correlations between Parameters of Traditional and Polyphenolic Maturity

Table 4 displays the coefficients of the linear correlation between the variables of traditional and polypehnolic maturity. Negative correlations were found in the case of three variables: weight of 100 berries, total potential polyphenols, and extractable polyphenols. The fact that the correlations are negative could be traced to the increase in volume of the grape berries, which results in a decrease in phenolic compounds through dilution,[17] as well as a decrease in the ratio of grape skin/total berry weight. Relatively small-sized berries are generally favored as higher quality, in part because smaller size equates to a higher ratio of skin to berry weight, resulting in a favorably high concentration of phenolic compounds in the resulting must and wine.[7] In addition, the higher correlation value obtained between the weight of 100 grape berries and the TPIpH3 (-0.8507) compared to that obtained with the TPIpH1 (-0.6053) indicated an influence of maturity on the ease of extraction of phenolic compounds, given that the extraction of substances present in the skin increased in direct proportion to the maturity of red grapes.[18] The grapes of largest volume in the case of this research were those whose berry weight was augmented by increased water intake and, accordingly, these berries matured later under equal conditions.[19] Based on the results obtained, this had a greater effect on the liberation capacity of phenolic compounds than on their synthesis and accumulation—the correlation values obtained with TPIpH3 were higher than those with TPIpH1, in spite of the fact that the dilution effect was the same for both cases.

The parameter °Brix presented significant correlations with all the variables that evaluate total phenolic compounds in grapes: TPIpH1, TPCLAM, and TPIpH3. In particular, the correlation level between °Brix and TPIpH3 was very high (0.9174), which demonstrated that sugar content, as well as the ease of extraction of phenolic compounds from skin, increased with the degree of maturity of the grape. Accumulation of a high concentration of sugar in grapes is a sign of good maturity, and generally also implies the concurrent accumulation of other quality elements, such as color,

TABLE 4
Lineal correlations between the parameters of traditional maturity and those of polyphenolic maturity

Variables	*Significance (%)*	*Correlation*
Weight 100—TPIpH3.2	95	−0.8507
Weight 100—TPIpH1	85	−0.6053
°Brix—TPIpH3.2	95	0.9174
°Brix—TPCLAM	95	0.7901
°Brix—TPIpH1	85	0.6545
pH—EA	95	−0.9037
pH—TA	95	−0.7793
TA—EA	95	0.7961

tannins, etc. A linear regression fit between BRIX and TPIpH3 variables yielded a moderately high (0.81) R^2 coefficient, low standard error (1.38), and an average percentage error (absolute value) for predictions of around 3.6%. These results suggest that through quantification of the sugar content of the must (°Brix), the concentration of total extractable grape polyphenols can be approximately predicted. This result also indicates that the parameter °Brix could serve as a good general indicator of grape maturity across samples, provided that the grapes are produced under similar climatic and viticultural conditions.

The pH variable in the obtained results was highly correlated (-0.9037) with the ratio of anthocyanin extractability (EA). EA expresses the difference between extractable anthocyanins and total anthocyanin content of the fruit, represented as the percentage of potentially extractable to total anthocyanins.[4,20] Depending on the variety and degree of maturity, extraction potential (extractability) can vary widely. This factor explains why grapes that are richer in anthocyanins do not always yield wines with more color.[21] The extractability of anthocyanins is a function of the state of maturity, specifically of the degradation of skin cells that takes place during berry maturation.[14] Therefore, as the grape matures, a decrease in EA can be observed that coincides with an increase in the breakdown of cell walls and an increase of pH due to the dilution, metabolism, and salting-out of acids. For this reason, and because cellular extractability (EA) is closely linked to variety,[22] a new linear regression fit was calculated, and in this case the variable "grape variety" was introduced in order to specifically take into account the influence of the variety. These characteristics are dependent upon many environmental factors as well, a particular case in point being that of Tempranillo, which in warm climates, or when grown in soils with high potassium content, will yield fruit with low acidity and high pH.[19] The model, and resulting equation, yielded two straight lines with slope and intercept varying according to the variety (Fig. 1):

$$\text{EA (variety Monastrell)} = 448.454 - 101,301^{*}\,(\text{pH grape}),$$

$$\text{EA (variety Tempranillo)} = 169.396 - 30.21^{*}\,(\text{pH grape}).$$

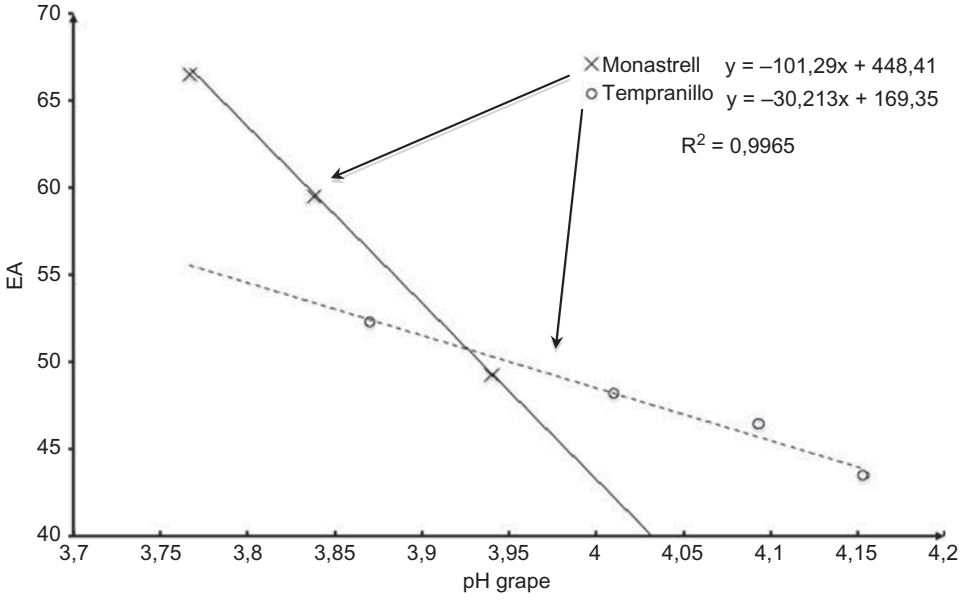

FIGURE 1 Lineal regression of extractibility (EA) against pH of grape and variety.

The correlation of the equation was very high ($R^2 = 0.9965$), and the average standard deviation, obtained by taking the absolute value of the calculated values with respect to experimental values, was less than 0.5%. The influence of variety on the prediction of extractability of anthocyanins was apparent. It was possible to infer that EA decreased more rapidly with the increase of pH in the Monastrell grape than in Tempranillo, in spite of the fact that the former had greater EA values at pHGRAPE values lower than 3.9. Thus, knowing the pH of the grape for the same variety, region and season, a good prediction of EA would be possible. However, given the wide variety of factors that can influence grape pH (excessive irrigation, potassium level, ratio of acids) predictions in this regard should be made with caution.

Finally, there was a moderately strong positive correlation (0.7961) between total acidity of the grape (grapeTA) and EA. The decrease in values of both variables as maturity advances, and, conversely, the prior increase in both values during earlier stages of maturation supports this relationship indicating that less mature grapes have a higher acid content and, likewise, a higher extractability ratio. Given the variable acidity level that has been observed across seasons,[17] which depends on variety and maturation conditions,[15] but which is principally a factor of varying malic acid content, the correlation value, and, particularly, the coefficients of the regression equation that relate both variables, may also vary significantly from season to season. Therefore, in principle, a given set of results will only be valid under conditions of the same variety, region, and season. In addition, the more localized the region in which the grapes are grown, and the more uniform the growing technique, the more precise the predictions based on the equation will be. Notably, no significant correlations were found between traditional maturity variables and those that evaluate the anthocyanin content of grapes, and only a weak correlation between pH and ANTpH1, attributable to the dilution effect of the must. However, this fact could be largely due to variance in the synthesis and accumulation of the anthocyanins as a result of adaptation of the vine to its particular soil and climate. The appearance of anthocyanins is clearly linked to the accumulation of sugars in grapes, but no direct relation could be established based on the results. Furthermore, it has been shown that parameters, such as light exposure, may augment the rate of accumulation of anthocyanins without affecting the sugar content of the skin.[17,23]

Correlations between Variables of Phenolic Maturity

Table 5 shows the correlations between all the variables associated with polyphenolic compounds and/or phenolic maturity. Based on the results, the anthocyanin content in grapes calculated through the Lamadon method (ANTLAM) showed high positive correlations with total polyphenols at pH1 (TPIpH1), total potential anthocyanins (ANTpH1), and potential extractable anthocyanins (ANTpH3). In addition, a negative correlation was observed with the ratio of phenolic maturity of seeds (Mp), although this correlation was weaker (-0.6064), and significant only if the significance level was reduced to 85%. The latter result agrees with that observed by Blouin et al.,[14] who reported that the tannin content of seed extracts decreases after veraison, and that this decline is associated with the accumulation of anthocyanins in skins.

A high correlation (0.8267) was observed between ANTLAM of the Lamadon method and the ANTpH1 of the Saint-Cricq method, the two variables that evaluate total (potential) content of anthocyanins. This suggests that both methods could be considered to offer comparable results in the calculation of total anthocyanin content in grapes. To further explore this result, an analysis of variance (ANOVA) was realized to determine if significant differences existed between the results given by the methods. The experiment designed for this purpose consisted of a three-level factor (VARIABLE) and seven blocks, corresponding to the seven grape samples. The characteristics of the design, in which the results of the variable ANTpH3 were also included, are shown in Table 6. The resulting ANOVA found no significant differences (LSD, 95%) between these two variables in the evaluation of total potential grape anthocyanins (ANTLAM and ANTpH1). However,

TABLE 5
Lineal correlations between the analyzed parameters in grapes

Variable	*Significance*	*Variable*	*Correlation*	P *value*
Variables for the Lamadon method (1995)				
ANTLAM	95	TIPpH1	0.8448	0.0167
		ANTpH1	0.8267	0.0218
		ANTpH3	0.7583	0.0482
	85	TPCLAM	0.6380	0.1231
		Mp	−0.6064	0.1489
TPCLAM	95	BRIX	0.7901	0.0345
		TIPpH1	0.7534	0.0505
	85	ANTLAM	0.6380	0.1231
		TIPpH3	0.6286	0.1306
Variables for Saint-Cricq de Gaulejac et al. method (1999)				
ANTpH1	95	ANTLAM	0.8267	0.0218
		TIPpH1	0.8037	0.0294
	90	ANTpH3	0.6827	0.0910
		pHGRAPE	−0.6754	0.0959
	85	EA	0.6540	0.1111
ANTpH3	95	Mp	−0.8492	0.0156
		ANTLAM	0.7583	0.0482
	90	ANTpH1	0.6827	0.0910
TIPpH1	95	ANTLAM	0.8448	0.0167
		ANTpH1	0.8037	0.0294
		TPCLAM	0.7534	0.0505
	85	BRIX	0.6545	0.1107
		PESO100	−0.6053	0.1498
TIPpH3	95	BRIX	0.9174	0.0036
		PESO100	−0.8507	0.0152
	85	TPCLAM	0.6286	0.1306
EA	95	pHGRAPE	−0.9037	0.0052
		TAGRAPE	0.7961	0.0322
	85	ANTpH1	0.6540	0.1111
Mp	95	ANTpH3	−0.8492	0.0156
	85	ANTLAM	−0.6064	0.1489

a significant difference *was* observed between these two variables and the potential of extractable anthocyanins (ANTpH3)—here, on average, a significantly lower value was seen across all grape samples for extractable, versus total, anthocyanins, as was expected.

Total phenolic compounds in grapes, calculated by the Lamadon method (TPCLAM), showed a moderately high positive correlation (0.7534) with total potential polyphenols (TPIpH1). The same trend was seen in the correlation with total polyphenols (TPIpH3), although with a much lower linear correlation value (0.6286), only significant at a significance level of 85%. The values of these three phenolic variables were compared by ANOVA according to the same approach used above for the anthocyanin variables. In order to be able to compare the three variables, the variable TPCLAM was transformed linearly by dividing by 0.08, to obtain a new variable (IPTLAM), which would represent the ratio of total grape polyphenols (DO_{280}) according to the Lamadon method. This follows as the calculation of total polyphenols originally made by the Lamadon method involved multiplying the ratio by 0.08. The ANOVA showed that there were no significant differences (LSD) between IPTLAM and TPIpH1 at a significance level of 95%, although the IPTLAM value was, on average, greater than that of TPIpH1 among the grapes studied. The origin of these differences could

TABLE 6
Statistical design for comparing variables that evaluate anthocyans in grapes

Sample	*Block*	*Variable*	*Anthocyans grape*	*Variable*	*Tip grape*
T1	1	ANTLAM	934.5	TIPLAM	50.1
T1	1	ANTpH1	780.5	TIPpH1	43.2
T1	1	ANTpH3	418.3	TIPpH3	29.0
T2	2	ANTLAM	829.5	TIPLAM	50.4
T2	2	ANTpH1	595.0	TIPpH1	39.2
T2	2	ANTpH3	338.3	TIPpH3	23.6
T3	3	ANTLAM	1099.9	TIPLAM	55.5
T3	3	ANTpH1	1090.3	TIPpH1	47.2
T3	3	ANTpH3	500.5	TIPpH3	31.0
T4	4	ANTLAM	900.4	TIPLAM	40.8
T4	4	ANTpH1	936.3	TIPpH1	40.2
T4	4	ANTpH3	484.8	TIPpH3	26.2
M5	5	ANTLAM	601.1	TIPLAM	28.2
M5	5	ANTpH1	735.0	TIPpH1	36.4
M5	5	ANTpH3	297.5	TIPpH3	24.0
M6	6	ANTLAM	669.4	TIPLAM	55.5
M6	6	ANTpH1	624.8	TIPpH1	41.4
M6	6	ANTpH3	316.8	TIPpH3	31.6
M7	7	ANTLAM	1115.6	TIPLAM	59.1
M7	7	ANTpH1	1165.5	TIPpH1	53.8
M7	7	ANTpH3	390.3	TIPpH3	27.4

be explained by the presence of ethanol (15%) in the extraction solution of the Lamadon method, in contrast to the solution used in the Saint-Cricq method. The extraction of tannins from seeds is favored by ethanol;[4,7] therefore, a greater quantity of tannins could be expected during extraction by the Lamadon method, thus resulting in a higher IPTLAM value with respect to TPIpH1. The value of the variable TPIpH3, the ratio of extractable polyphenols (phenolic abundance), was significantly different (95% significance) from IPTLAM and TPIpH1, which evaluate the ratio of total polyphenols. This was the same trend seen above among the grape anthocyanin variables.

With respect to both the anthocyanin and IPT results obtained by the two different methods, it should be added that variables in the Lamadon method are expressed in terms of kilograms of grapes, while those in the Saint-Cricq method are expressed in terms of liters of must. Assuming an approximate must-from-fruit yield of 75%, the variables of the Lamadon method should be multiplied by a factor of 1.33 to correct for this difference. Working with these corrected numbers, significantly higher values were obtained (95%) for the variables of the Lamadon method (ANTLAM and IPTLAM) than for those of the Saint-Cricq method (ANTpH1 and TPIpH1). Total grape anthocyanins values were comparable for both methods, while for variables associated with total phenolic compounds, the Lamadon method offered better results at a significance of 90%. This suggests that here the two methods are not exactly equivalent, an implication that is further supported by the fact that the polyphenolic variables are less strongly correlated than the anthocyanin variables.

Finally, it should be mentioned that the ratio of extractability of anthocyanins (EA) does not relate to any of the variables in the Lamadon method. This fact constitutes a disadvantage of this method, as it means that it can only be used to evaluate the phenolic content of grapes, while the Saint-Cricq method can be used to evaluate both the phenolic content and extractability of phenolic compounds contained in the grapes. Blouin et al.[14] affirm that methods that are sufficient to offer information regarding only the phenolic content of grapes are neither suitable for use in making accurate predictions of the phenolic content of the resulting wine, nor able to provide an estimate of the level of

phenolic maturity of grapes in order to determine harvest date. By this criteria, the Lamadon method is principally disadvantaged by its limitation for use only in predicting the phenolic content of wines through regression equations.

Significant Correlations between Wine Color/Polyphenol Content and Polyphenolic Maturity

The aim of this part of the research was to uncover relationships between wine color and phenolic content variables, and the variables associated with maturity. Table 7 presents linear correlation values between these variable sets. A strong positive correlation was found between anthocyanins in wine and extractable anthocyanin content in grapes (ANTpH3). This result coincides well with those of other authors, such as Saint-Cricq de Gaulejac et al.[4] and Blouin et al.[14] A strong degree of correlation was also seen between anthocyanins in wine and seed maturity (Mp), although here the correlation was negative (-0.8326). This result is in agreement with the strong inverse relationship between ANTpH3 and Mp discussed previously.

Anthocyanin concentration, as calculated by the Lamadon method, also evidenced a moderately strong positive correlation (0.7227) with the anthocyanin content in wine. Among the traditional variables of maturity, only grape total acidity was significantly correlated with wine anthocyanin

TABLE 7
Correlations between parameters analyzed in wine and grapes

Variable	*Significance*	*Variable*	*Correlation*	P *value*
WINE ANT	95	ANTpH3	0.8835	0.0083
		Mp	−0.8326	0.0201
	90	ANTLAM	0.7227	0.0666
	85	TAGRAPE	−0.6212	0.1365
TIP	95	TPCLAM	0.8704	0.0108
	90	ANTLAM	0.7218	0.0670
		TIPpH1	0.6623	0.1050
	85	BRIX	0.6190	0.1383
		TIPpH3	0.5867	0.1661
IND GEL	95	ANTLAM	−0.8953	0.0064
		ANTpH1	−0.8887	0.0075
		ANTpH3	−0.8047	0.0290
		Mp	0.7653	0.0450
	90	TIPpH1	−0.7297	0.0627
CI	95	ANTpH1	0.8927	0.0068
		TIPpH1	0.8546	0.0143
		ANTLAM	0.8255	0.0222
	90	pHGRAPE	−0.6702	0.0995
	85	ANTpH3	0.6245	0.1338
		TPCLAM	0.6003	0.1541
		BRIX	0.5998	0.1546
TINT	95	ANTpH3	−0.9109	0.0043
		ANTLAM	−0.9058	0.0050
		ANTpH1	−0.8626	0.0125
		Mp	0.7667	0.0443
	90	TIPpH1	−0.6986	0.0808

content (-0.6212). This finding is supported by the positive correlation between grape total acidity and extractability of anthocyanins, where grapeTA increased with increasing EA, and whereby the quantity of anthocyanins that passed to the wine decreased in accordance with the diminished proportion of extractable anthocyanins. The ratio of total polyphenols in wine (IPT) was strongly positively correlated with total grape phenolic compounds (TPCLAM) as calculated by the Lamadon method; the trend was similar, although the correlation less strong, between IPT and grape anthocyanins (ANTLAM) calculated by this method. Much weaker correlations were seen between variables analyzed by the Saint-Cricq method; the only significant correlation seen here, weak as it was (0.6623), existed between TPIpH1 and wine IPT.

The variable BRIX was weakly positively correlated (0.6) with the polyphenolic content in wine, as expressed by total phenolic index and C.I., a result which was in accordance with the observation that high sugar content in grapes is a sign of good maturation. The fact that the correlation value was low indicated, however, that traditional parameters of maturity provide an insufficient basis for confidently predicting the polyphenol content of wines.[24]

The gelatin ratio (INDGEL) evidenced moderately strong negative correlations with all the variables associated with anthocyanin content[25] in both methods, and particularly with anthocyanin content as determined by the Lamadon method[9] (ANTLAM). This suggests that high anthocyanin content in grapes will lend itself to an organoleptic softening of the wine, just as a decrease in tannin content in seeds, responsible for the astringency of wine, seems to be related to the accumulation of anthocyanins in skins.[14] This relationship of wine astringency to seed tannins, and to the gelatin ratio,[25] supported the positive correlation found between Mp, which evaluates the percentage of tannins in seeds, and INDGEL.

With respect to color intensity, strong positive correlations existed with total anthocyanin content variables, ANTpH1 (0.8927) and ANTLAM (0.8255), as well as with TPIpH1 (0.8546). The correlation with extractable anthocyanins (ANTpH3) was weaker (0.6245), suggesting that a closer relationship existed with total, versus extractable, content of anthocyanins and polyphenols. However, the use of pectolytic enzymes during vinification may explain these results. Finally, the tint parameter was strongly negatively correlated with all the variables that evaluated total and extractable anthocyanin content in grapes, and moderately strongly correlated with seed maturity.

Prediction of Wine Polyphenol and Color Character by Linear Regression Models

As a preface to making predictions about the different wine parameters, it should be reiterated that the sample set included only seven wines. Therefore, the results generated should serve only as a guide, since a greater number of samples would be necessary to formulate, and subsequently validate, a regression equation. To obtain a reliably sound regression equation would require preparing at least 30 wines according to a consistent vinification process, so as not to introduce additional variables that would bias the results.

Table 8 lists the regression equations relating the different parameters measured in the grapes to the polyphenolic measurements drawn from the wines. The linear regression model chosen to predict *anthocyanins* in wine, based on the classic maturation variables and those given by the Lamadon method,[9] was dependent upon two variables: total acidity of the must and the total anthocyanin content of the grape, calculated by the above-mentioned method. That obtained according to the phenolic maturity determination method developed by Saint-Cricq, on the other hand, depends on the total acidity of the must and potential extractable anthocyanins. By the Lamadon method, a greater difference between predictions and experimental values was observed, at a significance of 90%, for high values of (ANTLAM), from 900 mg/kg of grapes. These results indicate that predictions from the Saint-Cricq method are more accurate for grapes with a high total anthocyanin content. Thus, if it were necessary to choose between them, it would be advisable to opt for the latter method, although both offer reasonably similar results. An equation was proposed by

TABLE 8
Prediction of polyphenolic characteristics and wine color from analytical data for grapes

Prediction equations	R^2 *(%)*	*Typical error(%)*
ANTWINE = 659.78 - 65.46 * TAGRAPE + 0.16 * ANTLAM	86.65	3.2
ANTWINE = 553.98 - 40.34 * TAGRAPE + 0.41 * ANTpH3	87.11	3.2
TIP = 57.42 - 5.50 * TAGRAPE + 5.96 * TPCLAM	88.68	3.5
TIP = 19.58 + 1.96 * BRIX - 9.81 * TAGRAPE + 0.64 * TIPpH1	85.17	4.2
IND GEL = 73.95 - 0.028 * ANTLAM	76.19	7.1
IND GEL = 61.225 - 0.67 * TIPpH1 + 0.388 * Mp	94.96	3.5
CI = 16.31 - 2.31 * pHGRAPE + $2.4{\cdot}10^{-4}$ * ANTLAM	87.50	3.1
CI = 2.85 + 0.18 * BRIX + $2.3{\cdot}10^{-4}$ 9638 * ANTpH1	85.45	3.4
TINT = 0.67 - $1.2{\cdot}10^{-5}$ * ANTLAM	78.46	2.5
TINT = 0.58 - $1.5{\cdot}10^{-5}$ * ANTpH1 + $2.08{\cdot}10^{-4}$ * EA	96.90	1.0

Fernández-López et al.,[24] which has a slightly lower R^2 coefficient and which depends on the ratio of anthocyanin extractability (EA), on the Baumé degree of the must and on the anthocyanins and phenolic compounds calculated according to the Lamadon method.

The equation for making predictions on the *ratio of total polyphenols* in wine is straightforward and depends on total acidity of the must and total phenolic compounds in the grape as calculated by the Lamadon method. An equation is proposed by Kennedy et al.,[26] which, apart from the characteristic variables of the Lamadon method (anthocyanins and total grape phenolic compounds), depends on the °Brix, total acidity of the must, and TPIpH1. A good prediction of IPT can be made using either of the two methods, although a slightly better result is obtained with the variables calculated by the Lamadon method.

The equation for predicting the *gelatin ratio* using variables given by the Lamadon method depends on the anthocyanins in grapes as calculated by this method. The equation for prediction of the gelatin ratio with the variables from the Saint-Cricq method, on the other hand, depends on TPIpH1 and the maturity of seeds. In the prediction of the gelatin ratio, the Saint-Cricq method produces clearly superior results. This is due to the previously discussed fact that the Lamadon method, although it can satisfactorily make predictions about the ratio of polyphenols in a wine, is unable to make predictions relevant to its tannins.

For the calculation of *color intensity*, using the variables from the Lamadon method, the model is simple and depends on must pH and on grape anthocyanin content. The equation obtained by Fernández[20] depends on the tartaric acid content of the must, as well as on the content of anthocyanins and phenolic compounds calculated by the Lamadon method, and has an R^2 coefficient of 72.27%. The expression obtained by Kennedy et al.[26] depends only on pH and the tartaric acid content of the must and has an R^2 coefficient of 77.3%. Estimation of color intensity using variables given by the Saint-Cricq method, and traditional maturity variables, results in a simple equation that depends on the °Brix of the must and the concentration of anthocyanins calculated at pH 1.0. Thus, in this case, both methods provide reasonably approximate predictions of the color intensity of wine.

The equation for predicting *tint* depends only on anthocyanin content in grapes calculated by the Lamadon method. The inclusion of more variables does not improve the coefficient of the calculation. The expression obtained by García[27] and Pardo et al.[28] depends on the pH and total acidity of the must and its R^2 coefficient is higher (89.1%). The equation obtained for predicting wine tint using the Saint-Cricq method depends on the anthocyanin content calculated at pH 1.0 and on the skin cell maturity ratio. The results obtained by Fernández,[20] as well as in previous studies, indicate that good prediction results for wine tint are obtained through regression equations formulated based on maturity variables. Nonetheless, better approximations result from the equations that include variables from the Saint-Cricq method.

CONCLUSIONS

In this study, traditional determinants of maturity were not found to provide a sufficient basis for predicting phenolic composition in grapes and, as a result, also did not support determination of the optimum time for harvesting. This was due to the fact that correlations between the traditional variables of maturity and those which measure anthocyanins in grapes, which would permit such estimations, were not found to be sufficiently strong. With respect to the evaluation of anthocyanin content in grapes, both methods evaluated were statistically equivalent; a strong direct correlation was also seen between variables that measure total anthocyanin content in grapes and those that measure total polyphenolic compounds, although the latter relationship was weaker by the Lamadon method. The ratio of extractability of anthocyanins (EA) is unrelated to any of the variables in the Lamadon method. The fact that this method can only be used to evaluate phenolic content in grapes makes it much less preferable, in this regard, to the Saint-Cricq method, which evaluates content as well as extractability of phenolic compounds.

In the study of correlations between variables measured in grapes and the chromatic components of wine, a strong positive correlation was found between wine anthocyanin content and extractable anthocyanins in grapes. However, this correlation was weaker with anthocyanin values determined by the Lamadon method. On the other hand, the ratio of total polyphenols in wine was better correlated with the phenolic compounds as calculated by the Lamadon method than by the Saint-Cricq method. The gelatin ratio showed a strong negative correlation with all the variables that express anthocyanin content in grapes, and was directly correlated with phenolic maturity of seeds. And finally, the color intensity of wine was found to be closely and positively correlated with the variables associated with total anthocyanin content in grapes, as well as with total potential polyphenol content, and inversely correlated with the pH of the must.

REFERENCES

1. Flanzy, C. Oenologie fondements scientifiques et technologiques. Technique & Documentation Lavoisier; Paris, France, 1998.
2. Yildirim, H.K.; Elmaci, Y.; Ova, G.; Altu, T.; Yücel, U. Descriptive analysis of red wines from different grape cultivars in Turkey. International Journal of Food Properties **2007**, *10* (1), 93–102.
3. Di Stefano, R.; Cravero, M.C. Methods for determination of grape polyphenols. Rivista Viticoltura e di Enologia **1991**, *44* (2), 37–45.
4. Saint-Cricq de Gaulejac, N.; Vivas, N.; Glories, Y. Maturité phenolique: Définition et contrôle. Revue Françasaise d'Oenologie **1998**, *173*, 22–25.
5. Mandic, A.I.; Dilas, S.M.; Ćetković, G.S.; Čanadanović-Brunet, J.M.; Tumbas, V.T. Polyphenolic composition and antioxidant activities of grape seed extract. International Journal of Food Properties **2008**, *11* (4), 713–726.
6. Rababah, T.M.; Ereifej, K.I.; Al-Mahasneh, M.A.; Ismaeal, K.; Hidar, A.-G.; Yang, W. Total phenolics, antioxidant activities, and anthocyanins of different grape seed cultivars grown in Jordan. International Journal of Food Properties **2008**, *11* (2), 472–479.
7. Ferrer-Gallego, R.; García-Marino, M.; Hernández-Hierro, J.M.; Rivas-Gonzalo, J.C.; Escribano-Bailón, M.T. Statistical correlation between flavanolic composition, colour and sensorial parameters in grape seed during ripening. Analytica Chimica Acta **2010**, *660*, 1–2, 22–28.
8. Río Segade, S.; Rolle, L.; Gerbi, V.; and Orriols, I. Phenolic ripeness assessment of grape skin by texture analysis. Journal of Food Composition and Analysis **2008**, *21* (8), 644–649.
9. Lamadon, F. Protocole pour l'évaluation de la richesse polyphenólique des raisins. Revue des Œnologues **1995**, *76*, 37–38.
10. Boulton, R.G.; Singleton, V.L.; Bisson, L.F.; Kunkee, R.E. *Principles and Practiques of Winemaking*; Aspen Publication: Gaithersburg, MD, 1998.
11. Romero, R.; Zúnica, L. *Estadística. Proyecto de Innovación Educativa*; Servicio de Publicaciones de la Universidad Politécnica de Valencia: Valencia, Spain, 1992.

12. O.I.V. (Office International de la Vigne et du Vin). *Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts*; O.I.V.: París, France, 1990; 368.
13. Blouin, J. *Technique d'Analyses des Moûts et des Vins*; Dujardin Salleron: París, France, 1992.
14. Blouin, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. *Traite d'Œnologie. Tome II. Chimie du vin. Stabilisation et Traitements*; Dunod: Paris, France, 1998.
15. Blouin, J.; Guimberteau, G. *Maturation et Maturité*; Féret: Bordeaux, France, 2000.
16. Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Convaud, A. *Traite d'Œnologie. Tome I. Microbiologie du vin. Vinifications*; Dunod: Paris, France, 1998.
17. Jordão, A.M.; Ricardo Da Silva, J.M.; Laureano, O. Evolution of anthocyanins during grape maturation of two varieties (*Vitis vinifera* L.), Castelao Frances and Touriga Francesca. Vitis **1998**, *37* (2), 93–94.
18. Statgraphics Plus User Manual, Version 2.0 for Windows. Magnugisties Inc.: Rockville. MD, 1995.
19. Hidalgo, L. *Tratado de Viticultura General*; Mundi-Prensa, Madrid, Spain, 1999.
20. Fernández, J.I. Experiencias realizadas en la bodega experimental en la vendimia de 1998. *III Seminario Mejoras Técnicas en la Calidad Final de los Vinos*, Jumilla, June 17–18, 1999.
21. Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. American Journal of Enology and Viticulture **2001**, *52* (2), 67–86.
22. Vivas de Gaulejac, N.; Nonier, M.F.; Guerra, C.; Vivas, N. Anthocianin in grape skins during maduration of *Vitis vinifera* L. cv. Cabernet Sauvignon and Merlot Noir from different Bourdeaux terroirs. Journal International des Sciences de la Vigne et du Vin **2001**, *35* (3), 149–156.
23. Robin, J.P.; Sauvage, F.X.; Pradal, M.; Chovelon, M. Soil reflectance and colouring of grape. Vine red light excitation could be decisive for grape berry quality. Journal International des Sciences de la Vigne et du Vin **2000**, *34* (3), 101–119.
24. Fernández-López, J.A.; Hidalgo, V.; Almela, L.; López, J.M. Quantitative changes in anthocyanin pigments of *Vitis vinifera* cv Monastrell during maturarion. Journal of the Science of Food and Agriculture **1992**, *58*, 153–155.
25. Blouin, J.; Papet, N.; Stonestreet, E. Étude de la structure polyphénolique des vins rouges par analyses physico-chimiques et sensorielles. Journal International des Sciences de la Vigne et du Vin **2000**, *1*, 33–40.
26. Kennedy, J.A.; Troup, G.J.; Pilbrow, J.R.; Hutton, D.R.; Hewitt, D.; Hunter, C.R.; Ristic, R.; Iland, P.G.; Jones, G.P. Development of seed polyphenols in berries from *Vitis vinifera* L. cv. Shiraz. Australian Journal of Grape and Wine Research **2000**, *6*, 244–254.
27. García, T. Valoración del potencial fenólico de uvas Monastrell y su aplicación para predecir los parámetros del color de los vinos. Proyecto fin de carrera. E.T.S.I. Agrónomos de Orihuela, Universidad Miguel Hernández, 2000.
28. Pardo, F.; Garijo, J.; Alonso, G.L.; Salinas, M.R. Valoración del potencial polifenólico en uvas Monastrell. Predicción del contenido polifenólico de los vinos obtenidos. Viticultura/Enología Profesional **1999**, *61*, 57–66.